85. STRATEGIES FOR THE DETECTION OF UNKNOWN BIOLOGICAL MATERIALS

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ABSTRACT

Current strategies for the detection and identification of biological agents depend on known biological properties – specific biochemistries, specific antigens, and specific nucleic acid sequences. But what about unknown agents, either natural or man-made? Can these agents elude current or proposed detection/identification schemes? Are there strategies that can be implemented for detection?

Using the current template of trigger, detector, or identifier, strategies will be discussed that can be used to detect unknown materials of biological origin. Generic detection schemes may have a role in this area. Several recent efforts have investigated both the use of pathogen receptors, such as the Gm_1 ganglioside, or specific nucleic acid sequences, which are known as "islands of pathogenicity". Perhaps these types of approaches might be exploitable in future detection/identification efforts.

Detection and identification strategies, as they might be applied to the detection of unknown materials, will be reviewed as to speed, complexity, and information generated. Trade-offs among these parameters and the introduction of new detection and identification schemes in concert with current, proposed, or future technologies will be discussed.

The intent of this paper is not to solve the problems, but to provoke new ideas, so that effective biological detection capabilities for the 21st century can be developed.

INTRODUCTION

One of the keys to deterring the use of biological weapons is real time detection and warning. In the event that these agents are utilized, it is even more important to classify and eventually identify the type of agent so that appropriate countermeasures can be initiated. Several systems have been developed to provide detection and alarm of a biological agent attack. These first generation systems detect the characteristics of an aerosol in order to measure changes in the aerosol content against a background. This may be indicative of a man-made (not naturally-occurring) event that could indicate a possible attack with a biological agent. These first generation systems then use antibodies to provide a means of characterization of the aerosol for specific types of biological materials. This approach presupposes a knowledge of what an adversary may have in their arsenal. With the advent of genetic engineering and the potential proliferation of these techniques to design new types of agents that may defeat current detection and identification strategies, additional or alternative signatures need to be exploited to reliably indicate a possible biological attack. This paper explores some additional signatures that can be used in this context. Some approaches to improving medical intervention are also discussed.

Bio/non-Bio Determination

Parameter	Test	Rationale	Status
DNA/Protein Determination	Flow Cytometer	Unknown agents will probably be present in particles that have measurable protein/DNA.	Available(Field) - System used on several platforms. Taken out of JBPDS.
	Fluorometer	Same as above. Measures total flux not associated with particles.	Available(Field) - System demonstrated.
	Luminescence (intercalating peroxyoxalate esters)	Same as fluorometer.	Available (Lab) - Could be developed into a field system.
Heme Determination	Luminol Luminescence	Biological materials will have heme present.	Available (Field) - US BDWS Program.
Viability Determiantion	ATP Luminescence	Viable biological materials have ATP present.	Available (Field) - System demonstrated.
	Fluorescein Diacetate	Measures presence of lipases.	Available (Field) - Routine reagent used in FCM and fluorescence.

Currently particle size is the most widely used parameter that triggers an alarm, although technologies that measure particle shape, fluorescence from biological markers (tryptophan or NAD/NADH), or ATP luminescence are being actively developed. There are additional signatures that can be exploited. For example, elemental analysis can be performed to evaluate if a change in the ratio of various elements has occurred. Organic analysis can simultaneously be performed to evaluate if materials that are consistent with various propellants, encapsulants, and aerosol additives are indeed present. Changes in either the inorganic (elemental) or organic signatures could be highly indicative that a biological agent or agents have been used. These parameters can be used as an initial approach to determine if the background aerosol characteristics have changed; however, further characterization is warranted.

CHANGE IN BIOLOGICAL FLUX

For example, it might be useful to see if there is a dramatic change in the biological flux of the environment. This is currently performed on existing, such as the US Army's Biological Integrated Detection System (BIDS) units through the use of DNA measurements by flow cytometry and changes in the flux of Adenosine-Tri-Phosphate (ATP) by luminescence techniques. However, there are additional parameters that can be measured. For example, simultaneous DNA and Protein measurements have been shown to be effective to measure changes in the biological flux. Other parameters, such as heme measurements,

viability changes, and a version of the Gram stain may also prove to be useful.

PATHOGENICITY DETERMINATION

If a change in the bioflux is determined to be significant, the next step would be to determine whether this change is a biological material that is dangerous, i.e., may cause death or poses a threat to health. Thus we need to make a determination as to whether or not the material is a pathogen or a non-pathogen.

Various approaches can be used to make such a virulence determination. For example, many pathogenic materials bind to the Gm1 ganglioside, and this has been used as the basis for assays of several toxin materials for close to thirty years. More recently, DNA fragments, called aptamers, have been described, that can potentially be made to recognize specific pathogenic structures. Nucleic acid analysis may also be performed to detect specific nucleic acid sequences that could code for pathogenic markers. Siderophores have also been proposed to be used in this context. Lastly, assays can be performed for products of virulence plasmids, such as the determination of the PA component of *B. anthracis* toxin or some of the various YOP proteins of *Y. pestis*.

CLASSIFICATION OF AGENT

The final step in this detection process would be to classify the threat material as a bacterial, virus, proteinaceous toxin, or non-proteinaceous toxin. The most expedient way to make this determination could be through mass spectrometry. Pyrolysis mass-spectroscopy does have this capability, but there are some alternative methods that can be used. Bacteria can be determined through the use of a Gram stain and specific enzyme activities may prove useful. For example, beta-galactosidase is an enzyme that is widely used to detect and presumptively identify the presence of *E. coli* in water samples. Some other bacteria also possess similar activities.

In the context that these bacteria are used as weapons, one may assume that antibiotic resistance has been introduced into them. There are various tests that are currently available clinically to make these determinations. Most of them use a colorigenic substrate that measures a lytic enzyme, such as penicillinase. More current techniques could utilize nucleic acid probes to measure the presence of DNA sequences in plasmids or plasmid constructs that code for these lytic enzymes.

A similar approach can be taken to determine the presence of virus. For example, some viruses possess specific enzyme activities that can be measured. The neuraminidase of Influenza virus is such an example. The properties of this enzyme were studied and were exploited in a rapid assay for detection.

Since viruses are intracellular parasites, one may be able to assume that there would be carrier cells or culture components present concurrently with the virus. One might be able to use an assay for ovalbumin in cases where eggs are used as the carrier. If conventional cell culture is used as the means to grow the virus, the mitochondria from them might be measurable by using a fluorescent dye, such as Rhodamine 123. Histocompatibility antigens, which are species-specific antigens that are present on cell surfaces, may also prove useful as a means to detect the presence of viruses.

Proteinaceous toxins can be determined by several means. Conventional protein determination approaches, such as the Biuret, Coomassie Blue, and others, could serve as an initial screen. This could be followed by more stringent analysis, such as capillary electrophoresis and sequencing. This sequence could then be introduced into a bioinformatics tool, and a possible function could be determined. In the event that the toxin may have some

type of enzyme activity associated with it, substrates for the enzyme can be determined and used in subsequent analyses.

Some of the approaches described in the virulence determination section can also be used to determine if the toxin is dangerous to life and health. For example, the Gm1 ganglioside is found on many cells and many of the pathogenic toxins bind to it. Specific examples include SEB and cholera toxin.

The final type of potential weapon is the non-proteinaceous toxin. These materials would probably require mass spectrometry for detection and identification, although there are some alternative approaches. Live cell assays can be used to measure the effect of the compound on a cell, such as a neuron, and from this activity some information on its activity can be determined. Lastly, the current activity on bio-chips can also be exploited to detect these types of materials. It is conceivable that these chips can serve as substrates for receptor-based assays. An array of various receptor types can be coated to these substrates and used for detection.

From these approaches, detectors with improved capabilities to detect and warn may be developed that improve one's abilities to protect both troops and assets. As can be seen by these approaches, some of these technologies exist today and can be implemented into the field with some success. However, with the improvements in coating technologies and the integration of biological polymers with electronics, the next 50 years should see the development of detectors that utilize several of these approaches in concert.

IDENTIFICATION AND MEDICAL COUNTERMEASURES

Once a determination has been made in the field that a biological event has occurred, the next step would be to retrieve the suspect samples and return them to a lab for further identification and classification. There are a plethora of techniques that are available for identification, such as metabolic tests, carbohydrate or fatty acid analysis, phage typing, immunological assays, and nucleic acid probe technologies. Current identification techniques use rigorous analysis to identify microorganisms according to complex taxonomic schemes, using both DNA and RNA analysis. The degree of relatedness among genus, species, and strains can thus be determined. The use of chip technology, although now in its infancy, will play a crucial role in the future in these determinations.

RAPID ANTIBIOTIC SUSCEPTIBILITY TESTS

A current rationale, that one needs to know the identity of the particular agent so that the proper treatment modality can be employed, needs to be re-evaluated. In classical medical approaches, when one knows the identity of the organism, one can typically prescribe the appropriate course of antibiotics or other therapies. However, with the advent of genetic engineering and the relative ease that this allows an adversary to impart resistance to antibiotics, one can no longer assume that the mere identification of the organism would be sufficient for treatment. Even in conventional medical approaches, there has been an increase in the use of susceptibility tests to determine the appropriate course of treatment. In the case of an intentional release of a biological agent by an adversary, the use of antibiotic susceptibility testing is crucial.

In the event that a biological attack has occurred, particularly with a bacteria or a toxin, a viable sample is crucial for initiation of medical countermeasures. Although koch's postulates will have to be demonstrated for legal purposes, the viable sample will be necessary so that antibiotic susceptibility testing can be performed. Currently these tests involve isolating the organism and eventually obtaining it in pure culture for further analysis. In the case of a bacterium, the kirby-bauer procedure is usually used and takes 8 or more

hours to complete. In the event of a biological attack, there may not be sufficient time to do this. What is needed is a rapid method to do these determinations on environmental samples.

Several approaches have been proposed to approach this. One utilizes flow cytometry and rapid determinations in 20 minutes have been achieved. This approach uses a classical approach where the viable cells are mixed with an antibiotic mixture. The effect of the antibiotic on the cells is then measured by changes in scatter or DNA-specific dye uptake. Alternatively, analysis with nucleic acid probes that are specific for sequences that code for antibiotic resistance can be used. This has the added advantage over conventional techniques in that viable samples are not required.

A similar paradigm can be assumed for viruses, but toxins are a different case. Specific identification needs to be obtained so that the proper antidotes, if available, can be administered. In the case of real unknown entities, bio-informatics will eventually play a role so that the possible physiological activity of the material can be obtained. However, we are several years away from this being a field consideration.

IMMUNE STATUS DETERMINATIONS

Up until this point, the agents themselves have been discussed; however, the other part of the equation is the troops that have been attacked. It may be desirous to make a determination as to who has been attacked so that the field commander may not have to compromise his military posture. One possibility is to measure the status of immune function of the individuals who were involved in the attack. There are several approaches that may be used to assess immune status, from the classical blood cell counts to measuring individual lymphocyte status by flow cytometry analysis. As our ability to measure these functions improves, they may prove to be viable assets in the field.

DEPLOYMENT STRATEGIES

The previous discussion dealt with the types of technologies that one could conceivably use to detect the presence of biological materials; however, concepts of employment still need to be determined. This is the difficult part because it is here where the considerations are more cost/benefit, logistics, and personnel driven, rather than science. If we were to determine the types of scenarios that one would get in a potential terrorist scenario, there would be 2 cases: high value, fixed assets (buildings, stadiums, etc.) and hoax scenarios. In both of these cases, the scenarios are quite different. Another consideration is responding post attack. Here we need to determine of areas are contaminated and then perform quality control on personnel and materiel decontamination.

If there are some high value, fixed assets where a threat is credible, then some type of continuous monitoring system is probably worthwhile considering. These types of systems could utilize one or more of the triggering/detection technologies. For example, particle size and shape analysis coupled with fluorescence, could be an effective system. Systems such as the CDC 4WARN or the BAWS system under development in the US could be likely candidates. To minimize false alarms, this approach could be coupled with a detector system that utilizes some of the detector schemes, such as bio/non-bio or pathogen/non-pathogen. An ideal candidate for this type of approach is a flow cytometer since it can couple several of these parameters in one platform (Particle size, shape, bio/non-bio, viable/non-viable, pathogen/non-pathogen) in one platform. However, the down side of this approach is that it would require some degree of maintenance by building personnel.

Hoax scenarios can be dealt with by relatively simple equipment. One initially needs to make a bio/non-bio determination, and if positive by say a DNA test or protein test, a viability test could then follow to determine if live materials are indeed involved. Samples

can then be further analyzed by identification technologies, either on or off site.
Responding to an attack, however, requires an echelon of response. One first needs to determine if there is indeed a biological agent present. This can be determined by the same means discussed in the hoax scenario. If it is determined that indeed live biological material is used, then the area can be sampled to determine the extent of contamination. Lastly, the area can then be decontaminated and the effectiveness of this process can be determined by several simple tests, such as luminescence.

CONCLUSIONS

From this brief discussion, it can be shown that the problem is not insurmountable; however, several things need to change. We first need to become aware that the possibility does indeed exist that "unconventional" biological agents be encountered in the field. There are a variety of strategies that could be implemented in either trigger or detection platforms that could be used to detect signatures from biological agents and possibly determine that they could present a danger to health and life.

However, we need to change our paradigms on how we think about the problem. The most important thing is to provide detection and warning system that exploits a credible signature so that those in peril can take the appropriate protective measures.

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